SYNTHESIS AND BIOTECHNOLOGICAL STUDY OF ETHYL 2-((*E*)-2-((*E*) PHENYLALLYLIDENE) HYDRAZINECARBOTHIOAMIDO)ACETATE

SINTEZA ŞI STUDIUL BIOTEHNOLOGIC AL 2-((*E*)-2-((*E*) FENILALILIDEN) HIDRAZINCARBOTIOAMIDO)ACETATULUI DE ETIL

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The quality and length of human life is directly related to the development of medicine, in particular drugs. The modern world is constantly in the process of developing new, more effective drugs. Thiosemicarbazones may be one of the promising solutions. Despite their outstanding biological activities, many of them are toxic and cannot be used as medicines. The solution may be thiosemicarbazones, which are based on natural substances. This is expected to significantly reduce toxicity without impairing the biological activity of the substances. In this regard, for this work, a thiosemicarbazone was synthesized, which is based on two natural substances, glycine and cinnamaldehyde. To test the toxicity of this substance, some strains of cyanobacteria were used in this article. In addition to the lack of toxicity to these microorganisms, the synthesized thiosemicarbazone had a biostimulating effect.

Keywords: *thiosemicarbazone, cyanobacteria cultivation, non-toxic, cinnamaldehyde.*

Calitatea și durata vieții umane sunt direct legate de dezvoltarea medicinei, în special a medicamentelor. Lumea modernă este în mod constant în curs de dezvoltare a unor medicamente noi, mai eficiente. Tiosemicarbazonele pot fi una dintre soluțiile promițătoare. În ciuda activităților lor biologice remarcabile, multe dintre ele sunt toxice și nu pot fi utilizate ca medicamente propriu-zise. O soluție în acest sens poate fi tiosemicarbazonele care au o proveniență naturală. Se poate aștepta ca această funcționalizare pentru tiosemicarbazone să reducă semnificativ toxicitatea și să stimuleze efecte sinergetice, fără a afecta activitatea biologică a substanțelor. În acest sens, pentru această lucrare a fost sintetizată o tiosemicarbazonă, care are la bază două substanțe naturale, glicina și cinamaldehida. Pentru a testa toxicitatea acestei substanțe, în acest articol au fost folosite câteva tulpini de cianobacterii. Pe lângă lipsa de toxicitate față de aceste microorganisme, tiosemicarbazona TSC a avut un efect biostimulator impresionant.

Cuvinte-cheie: *tiosemicarbazona, cultivarea cianobacteriilor, netoxice, cinnamaldehida*

INTRODUCTION

A person's life expectancy largely depends on the quality and development of medicine in the region of his residence. A huge part of it is made up of drugs. In this regard, the development of methods for the synthesis and extraction of biologically active substances has a great interest. For quite a long time it has been believed that thiosemicarbazones are a promising area of modern research in the field of creating new, effective medications. A wide range of biological activities, such as anticancer [1], antimicrobial [2], antitumor [3], and antifungal [4], distinguishes this class of organic substances. However, their use is currently very limited. This is due to the high toxicity of many of them, or due to the lack of the desired therapeutic effect. The structure of thiosemicarbazones is strongly related to their toxicity and efficacy. This determines the need to develop new methods for selecting substances that will be synthesized in the future. In addition, new methods for testing the toxicity of synthesized substances are needed. At the same time, it is worth looking for new applications of thiosemicarbazones.

The goal of this work is the synthesis of a new thiosemicarbazone and its physicochemical and biological study. Taking into account the previously listed facts, thiosemicarbazone was synthesized based on two natural substances – glycine (as primary amine, in ethyl ester form) and cinnamaldehyde (as aldehyde). Glycine is the most important and the simplest amino acid. Cinnamaldehyde is a non-toxic, natural compound, which is found in the bark of cinnamon tree. The next step was to test thiosemicarbazone on cyanobacterial strains. These microorganisms are responsible for most of the oxygen produced on Earth. In addition, they can be used as raw materials for the extraction of phycoerythrin and phycocyanin, as well as in the cosmetic and pharmaceutical industries [5-6]. Phycobiliproteins contained in cyanobacteria have a strong antioxidant effect. Nutritional supplements based on them can slow down aging [7]. Phycobiliproteins are also used in immunofluorescence analysis [8]. Testing thiosemicarbazone on cyanobacteria will assess its toxicity. It is also known that some antibiotics can stimulate the growth of cyanobacteria biomass [9]. This means that a similar effect can be expected with thiosemicarbazone.

MATERIALS AND METHODS

Materials

The scientific research took place within the "Advanced Materials in Biopharmaceuticals and Technic" and "Ficobiotehnology" Scientific Research Laboratories of the State University of Moldova. The syntheses were performed with reagents purchased from the companies "Sigma-Aldrich", "Acros Organics" or "Alfa Aesar", being used in the synthesis without an additional purification. The cyanobacteria strains used in research take part of the Non-pathogenic Microorganisms Collection of the "Ficobiotehnology" scientific research laboratory.

Synthesis procedure

The desired thiosemicarbazone was synthetized using the same procedure, described in [10]. Primary amine was used as starting material for obtaining thiosemicarbazides. For this scope, primary amine was thiophosgenated to form isothiocyanate. In the next stage, the addition of isothiocyanate to hydrazine monohydrate resulted formation of thiosemicarbazide.

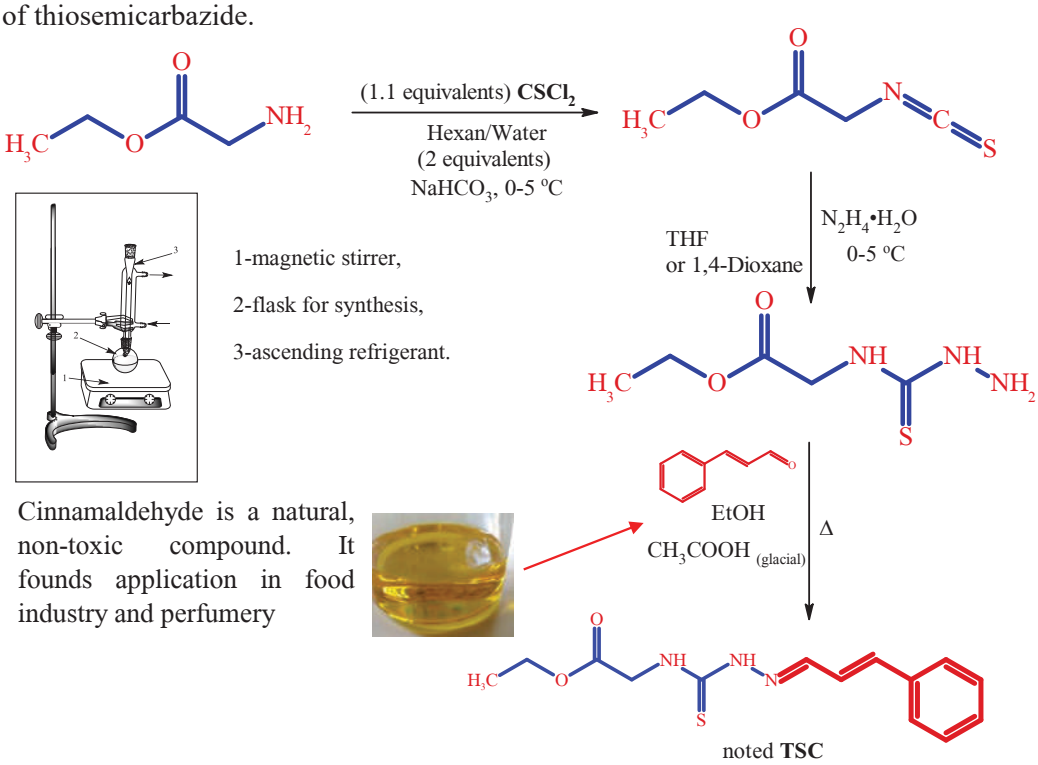

Figure 1. Synthesis of ethyl 2-(2-(3-phenylallylidene)hydrazinecarbothioamido)acetate

Synthesis of ethyl 2-isothiocyanatoacetate

Ethyl 2-aminoacetate and $NaHCO_3$ were dissolved in water. Thiophosgene in hexane was added to the obtained solution, with constant stirring. The reaction mixture was cooled in an ice-salt bath. After the addition of thiophosgene, the reaction mixture was stirred at the room temperature for 3 hours. Next, the hexane layer was separated and washed 3 times with saturated $NaHCO_3$ solution and 3 more times with distilled water. Obtained solution was dried with anhydrous Na_2SO_4. After, the solution was concentrated by distillation and purified by flash column chromatography. The hexane-ethyl acetate (2:1) solution was used as the eluent. Pink oil was obtained, yield 70-75%.

Synthesis of 2-[(aminocarbamothioyl)amino]ethyl acetate

To an alcoholic solution of hydrazine monohydrate, with constant stirring, an alcoholic solution of ethyl 2-isothiocyanatoacetate was added dropwise. The reaction mix-

ture was cooled in an ice bath. The pink solid was precipitated. Then, the mixture was stirred for 30 minutes at room temperature. The obtained solid was filtered, washed with cold ethanol and dried. Obtained: pink solid, 88-90% yield.

Synthesis of ethyl 2-(2-(3-phenylallylidene)hydrazinecarbothioamido)acetate

The cinnamaldehyde and 2-[(aminocarbamothioyl)amino]ethyl acetate were dissolved in ethanol. Glacial acetic acid was added as a catalyst (3-5 drops). The mixture was refluxed for 2 hours. After cooling the precipitated solid was filtered, washed with cold ethanol and dried. Obtained: brown solid, yield 85-86%. Throughout this work, the abbreviation TSC will be used for this substance.

Cyanobacteria cultivation and supplementation

The cyanobacteria of *Nostoc halophilum* and *Calotryx marchica* strains biomass was cultivated using BG11 cultivation medium balanced with macro - and microelements. Supplement solution was dissolved directly in incubator to establish the desired concentration.

ADMET parameters calculation and visualization

ADMET parameters were calculated and visualized with SwissADME [11].

RESULTS AND DISCUSSION

Synthetized substances

The desired thiosemicarbazone was synthetized in good yield as well as him intermediates. The main functional groups of synthetized substances were confirmed by the FTIR spectroscopy, which indirect confirms their structure. Purity of substances was monitored by thin layer chromatography.

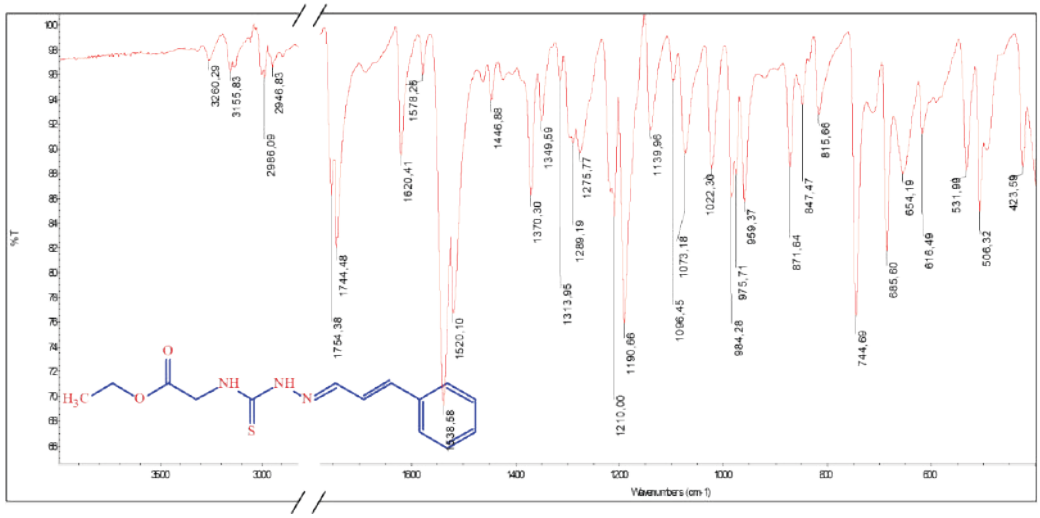

Figure 2. FTIR spectrum of ethyl 2-(2-(3-phenylallylidene)hydrazinecarbothioamido)acetate (TSC)

Also, for synthetized thiosemicarbazone were calculated ADMET properties. The assessment of these properties is very important for organic substances that can potentially be used for medical purposes. With their help, can be obtained approximate data on the solubility of a substance, his toxicity and permeability of biological membranes.

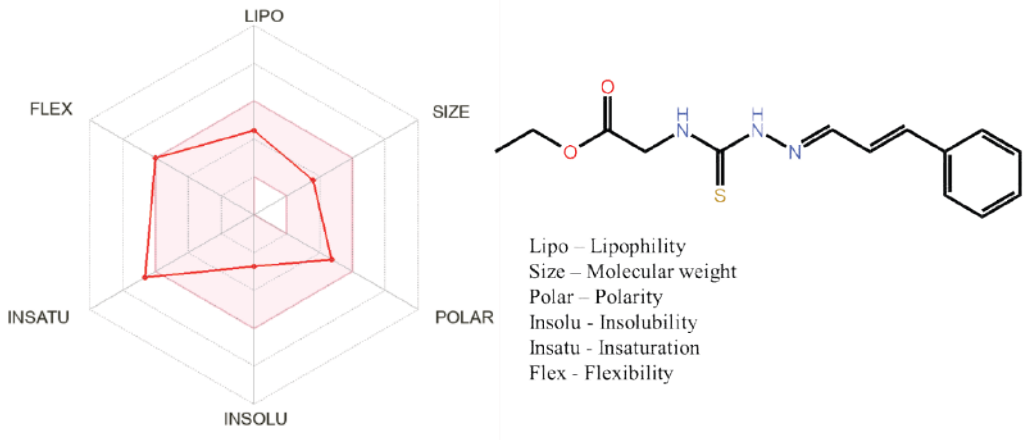

Figure 3. Diagram showing the main ADMET parameters of thiosemicarbazone. The optimal boundaries for a substance to be biologically active are indicated in pale red. The red line shows the parameters of the synthesized thiosemicarbazone

As we can see from the Figure 3, almost all parameters of the substance are in the zone of optimal values. The only slight deviation can be seen in the insaturation graph. This parameter depends on the fraction of sp^3 carbon atoms. From Table 1 can be seen that in the synthesized thiosemicarbazone it's only 0.21, while the optimal values are in the range from 0.25 to 1, which means that the proportion of saturated carbon atoms in the substance is too small. But, despite this deviation, the synthesized thiosemicarbazone fully complies with Lepinski's rule.

Table 1

Physicochemical properties calculated for TSC

Physicochemical Properties					
Fraction Carbon sp^3	Num. rotatable bonds	Num. H-bond acceptor	Num. H-bond donors	Molar Refractivity	TPSA, Å^2
0.21	9	3	2	83.74	94.81

It was found that the substance may have high gastrointestinal absorption. Also, the substance will most likely not be able to pass the blood-brain barrier (Table 2). It is important to note that this thiosemicarbazone is not a P-Gp substrate. This protein, also known as **multidrug resistance protein 1 (MDR1),** is responsible for the transport of

many substances in the body, such as lipids, peptides, and bilirubin. From its name can be understood that this protein can have a negative effect on the therapeutic effect of those drugs with which it interacts. This protein appears to severely limit the cellular uptake of drugs that are its substrates [12]. It was found that the synthesized thiosemicarbazone is probably not a substrate for P-Gp.

Table 2

Pharmacokinetic properties calculated for TSC

Pharmacokinetic Properties		
Gastrointestinal absorption	Blood–brain barrier permeability	P-glycoprotein substrate (P-Gp)
High	No	No

An Electrostatic Potential Map was generated for the synthesized thiosemicarbazone – **Figure 4**. It shows the distribution of partial charges (partial displacements of electron densities) in the TSC. Knowing this, we can determine the location of the most reactive atoms in the molecule. For example, the nitrogen atom from the azomethine group has a strong partial negative charge. Perhaps this enhances the donor properties of this atom, at least this will explain the relative ease of formation of coordination compounds by thiosemicarbazones precisely through this nitrogen atom, which is confirmed by a huge number of studies in this area, including using single crystal X-ray diffraction, like in [10].

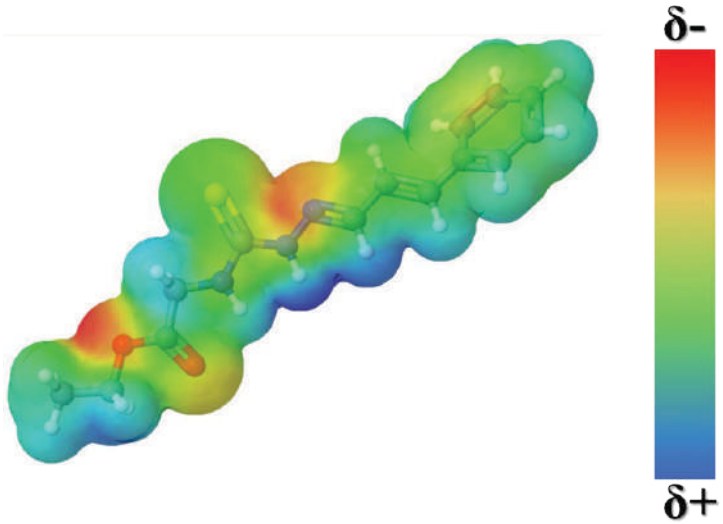

Figure 4. Electrostatic Potential Map of TSC (visualized with jmol)

Supplementation of cyanobacteria with TSC

To determine the optimal concentration, the *Nostoc halophilum* strain was cultivated with supplement in concentration diapason from 1 to 5 mg/L. Initially, 0.2 g of biomass was added to each incubator. Biomass was cultivated for 10 days. From Figure 3 we can observe that best concentration of the supplement for the accelerated development of the *Nostoc halophilum* is 2 mg/l. The biomass growth was stimulated almost 3 times compared to the blank sample and 4 times compared to the initial biomass.

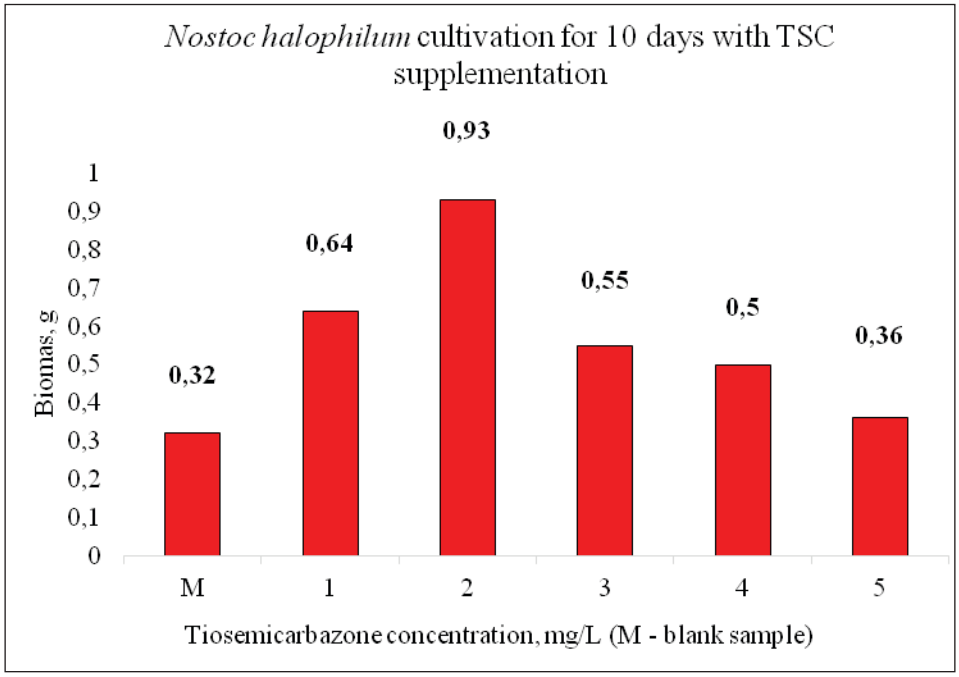

Figure 5. *Nostoc halophilum* cultivation for 10 days, with supplement 1-5 mg/L concentration diapason

In general, supplementation with TSC resulted in stimulation of *Nostoc halophilum* biomass growth at all additive concentrations. After this, the experiment was repeated for *Calotrix marchica* strain. From the Figure 4 follows that, as in the previous case, TSC was not toxic to *Calotrix marchica*. A significant growth stimulation effect is also noticeable. A concentration of 3 mg/L was optimal for accelerated cultivation of this strain. The biomass growth was stimulated almost 2 times compared to the blank sample and 4 times compared to the initial biomass.

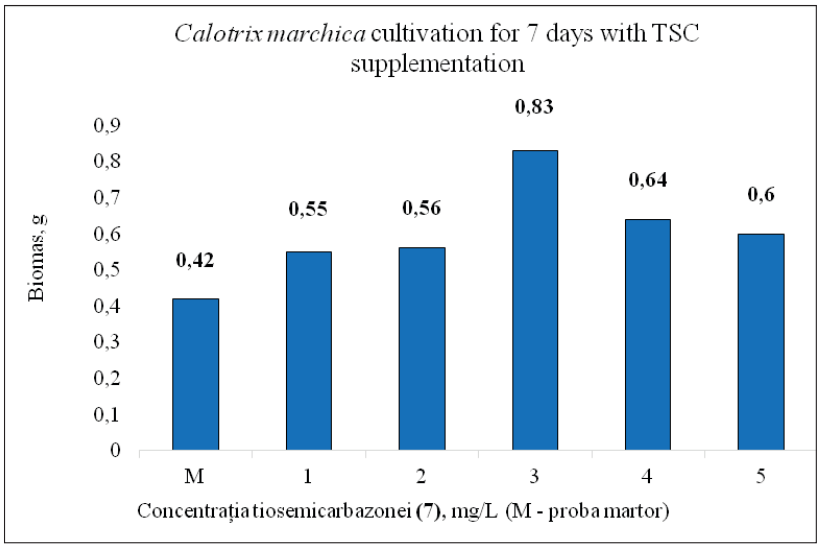

Figure 6. *Calothrix marchica* cultivation for 7 days, with supplement 1-5 mg/L concentration diapason

Phycobiliprotein extracts were prepared from the obtained biomass. They were analyzed using UV-vis spectroscopy. The Figure 5 represents the absorption spectrum of one of the obtained extracts. As you can see, in the region from 450 to 780 nm, two peaks are noticeable with maxima at 565 and 621 nm. They belong to phycoerythrin and phycocyanin, respectively.

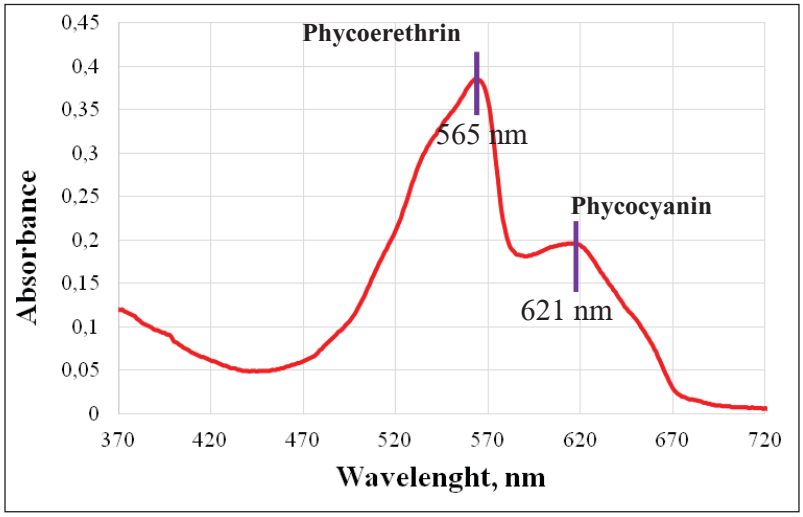

Figure 7. The electronic absorption spectrum of the extract obtained from *Nostoc halophilum*, after supplementation with TSC

CONCLUSIONS

Ethyl 2-(2-(3-phenylallylidene)hydrazinecarbothioamido)acetate (TSC) and its intermediates were obtained in good yields (70-90%). The functional groups of the synthesized substances were confirmed by FTIR spectroscopy. Purity was monitored by thin layer chromatography. Pharmacokinetic calculations were carried out, which showed that the synthesized thiosemicarbazone is in optimal values in order to have biological activity.

The biomass of *Nostoc halophilum* and *Calothrix marchica* cyanobacteria strains was cultivated with supplementation with ethyl 2-(2-(3-phenylallylidene)hydrazinecarbothioamido)acetate. As a result, it was found that the synthesized thiosemicarbazone is not toxic to these microorganisms and, on the contrary, has a good biostimulating effect. Optimal concentrations corresponding to the best growth-stimulating effect were established - for Nostoc 2 mg/L, for Calothrix 3 mg/L. Phycobiliprotein extracts were also obtained. They were examined using UV-Vis spectroscopy, which confirmed the presence of phycoerythrin and phycocyanin.

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